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Progressive vs Non-Progressive Onset of Chronic GVHD After ATG Prophylaxis Is Highly Predictive of Outcome

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The NIH chronic graft versus host disease (cGVHD) consensus statement emphasizes the importance of distinguishing acute from cGVHD based on clinical features rather than time. The proposed assessment of cGVHD severity appears to be prognostically important, but the relevance of categories and type of onset remains controversial. We retrospectively analyzed a homogeneously treated population of 600 patients transplanted between 1999-2010 with fludarabine 50 mg/m^2 daily X 5, IV busulfan 3.2 mg/kg daily x 4, ATG (Thymoglobulin, Genzyme - total dose of 4.5 mg/kg). An additional 287 patients (48%) received additional TBI 200cGy x 2. In this cohort, 245 patients (47%) had non-progressive cGVHD (NP-cGVHD) arising either *de novo* (n = 221) or after stopping primary immunosuppression (n = 24). In 71 patients (12%) progressive cGVHD (P-cGVHD) arose during systemic therapy for grade II-IV acute GVHD and 284 (41%) had no cGVHD. Patient and transplant factors were similar across all groups. Median followup was 84 (range 22- 161) months. The 5-year non-relapse mortality was 40% for patients with P-cGVHD compared with 11% for NP-cGVHD ($P < .0001$) and 12% for no cGVHD ($P < .0001$). Patients with no cGVHD had more relapse (38%) than those with P-cGVHD (28%,$P = .02$) and P-cGVHD (27%,$P = .002$). Overall survival for patients with no cGVHD was 61% vs 50% for P-cGVHD ($P = .005$) and 69% for NP-cGVHD ($P = .02$). Disease free survival was 54% vs 43% ($P = .003$) and 65% ($P = .003$), respectively. The median time on systemic immunosuppression for the 174 NP-cGVHD patients (71%) who required treatment was 273 days versus 398 days for all patients with P-cGVHD (p = ns). In conclusion, we propose that this simple means of classifying cGVHD is highly predictive of important transplant outcomes. It remains important both to prevent acute GVHD (and thus P-cGVHD) and yet take advantage of the graft-vs-malignancy effect conferred by NP-cGVHD.

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Glutathione Redox Metabolomic Dysregulation Precedes TNF-α Elevation and Predicts Severity of GVHD in Experimental Transplantation

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Graft-versus-host disease (GVHD) is characterized by cytokine and chemokine dysregulation that predicts and also contributes to disease severity. Intermediates in sulfur amino acid (SAA) metabolism are known to modulate cytokines implicated in GVHD, such as TNF-a and IL-2. In this first report, plasma and hepatic SAAs and other amino acid metabolite concentrations were sequentially profiled using redox metabolomics assay, in experimental allogeneic bone marrow transplantation (Allo-BMT) models and compared to syngeneic (Syn) BMT. At day +4, prior to increase in plasma TNF-a and subsequent GVHD histopathological changes, a significant decline in both plasma and hepatic glutathione (GSH) concentrations occurred resulting in a more oxidized redox potential in Allo BMT (Balb/C® B6), relative to Syn BMT (B6Thy1.1® B6) mice. The plasma free GSH concentrations in allogeneic mice were significantly lower (~65%; $P < .005$) when compared to Syn BMT controls and were accompanied by ~ 3 fold increase ($P = .012$) in plasma GSSG. Paradoxically, the plasma concentration of total cysteine, which is the rate-limiting substrate for GSH synthesis, was significantly higher in Allo BMT versus Syn BMT mice at day +10 suggesting that loss in GSH was not due to substrate limitation. Significant difference in the GSSG/GSH ratio was also noted in Allo / GVHD+ compared to Allo/GVHD- in un-irradiated paternal to F1 hybrid transplantation model (B6 ® B6D2F1), indicating that GSH depletion was a metabolic event associated with GVHD, and not a consequence of conditioning regimen (0.3150 ± 0.06007 vs 17.00 ± 4.907, respectively, p=0.0145). At day+10, metabolomic profile was able to segregate Allo/GVHD+ from Allo/GVHD- based on hepatic total GSH, free GSH and total cystinylglycine concentration. The plasma TNF-a elevation lagged behind redox changes with no significant difference detected in Syn-BMT and Allo-BMT group at day +4. Hepatic GCLC mRNA abundance, regulated by Nrf2 transcription factor, was decreased by ~50% ($P < .0001$) at day +4 and further declined ~80% by Day +10 ($P < .0004$), in Allo BMT compared to Syn BMT. Interestingly, hepatic nuclear Nrf2 and TNF-a concentrations at Day +10 were inversely correlated (r^2=0.8,$P = .01$) in Allo-BMT. Modulation of GSH in mixed lymphocyte culture using GSH depleting agents, buthionine sulfoximine and diethyl maleate, increased TNF-a expression in CD4 T cells compared to untreated controls ($P < .001$). These experiments show the utility of redox metabolomics to identify novel putative biomarkers in GVHD.

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A First-in-Disease Trial of in Vivo Costimulation Blockade for GVHD Prevention: The Addition of Abatacept to Standard GVHD Prophylaxis Controls Early CD4+ T Cell Proliferation and is Associated with Low Rates of Severe Acute GVHD

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We have shown that costimulation blockade can protect against acute GvHD (aGvHD) in a non-human primate model. We therefore designed a first-in-disease trial of *in vivo* CD28:CD80/86-directed costimulation blockade with CTLA4-Ig (abatacept) to prevent aGvHD after unrelated-donor HSCT for patients > 12y. In this trial, 10mg/kg abatacept was administered IV on day -1, +5, +14, +28 in addition to standard prophylaxis with cyclosporine + MTX.

Patient Characteristics and Survival: 10 patients, with a median age of 44.5 y (17-74) were enrolled and treated. 6 received 7/8-matched and 4 received 8/8-matched URD HSCT. 8 received PBSCs and 2 received BM. All received high-intensity conditioning. With a median follow-up of 367 days (262-680), 7 patients are alive and in remission and 2 patients died of relapse. One patient died, in remission, of trauma-related multi-organ failure > 1yr post-transplant.

Feasibility, Pharmacokinetics and Pharmacodynamics: All 10 patients received all scheduled abatacept doses, without infusion reactions. The average peak (230.9 +/- 7.4 mg/ml) and trough (45.9 +/- 2.8 mg/ml) abatacept levels, as well as the terminal $T_{1/2}$ (19.6 +/- 1.9 days) were similar to healthy controls. Importantly, as has been established *in vitro,* patients receiving abatacept demonstrated significant inhibition of post-transplant CD4+ T cell proliferation (with >80% reduction of Ki-67+ proliferating CD4+ T cells at d +28 compared to controls not receiving abatacept, Figure 1A).

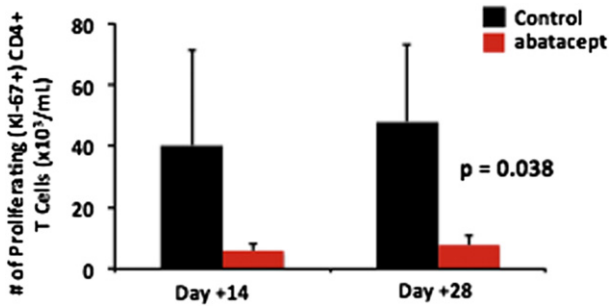

Figure 1A. Patients Treated with Abatacept Exhibit Fewer Proliferating CD4+ T Cells After Transplant. Black: Control patients treated with cyclosporine/MTX alone (n=6), Red: Patients treated with cyclosporine/MTX + Abatacept (n = 10)

Engraftment: All patients achieved neutrophil engraftment (median d+16.5) and donor engraftment (100% CD33 chimerism at d+30). Lymphocyte recovery was rapid: Day +100 counts showed ALC = 1053 +/- 259 cells/ml, total T cells = 741 +/- 208 cells/ml, and CD8+ T cells = 381 +/- 99 cells/ml. The Day +100 CD4+ T cells = 285 +/- 105 cells/ml, not significantly different from historical controls (n = 43) that received CNI/MTX aGvHD prophylaxis without abatacept (262 +/-26 cells/ml).

GvHD: Patients receiving the abatacept-containing regimen had encouragingly low rates of early severe aGvHD: Two patients developed aGvHD before day +100, with one of these patients (Gr II) progressing to steroid-dependent cGvHD of the liver and one patient (Gr III) with resolution of aGvHD. The cumulative incidence of grade II-IV and III-IV aGvHD by day +100 was thus 20% and 10%, respectively (Figure 1B). Importantly, there was no Gr IV aGvHD, no patient received salvage therapy for aGvHD, and there were no deaths from aGvHD.

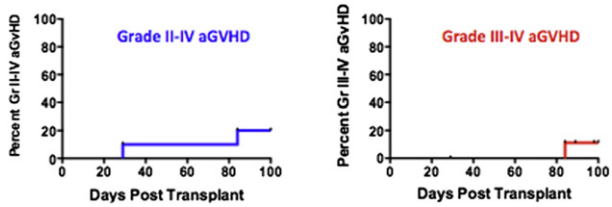

Figure 1B. Cumulative Incidence of Grade II-IV aGvHD (left) and Gr III-IV aGvHD (right) In Patients Through Day +100 on the Abatacept Trial. No Grade IV aGvHD developed.

Conclusions: This trial demonstrates, for the first time, the feasibility of adding *in vivo* T cell costimulation blockade with abatacept for aGvHD prevention. The decreased CD4+ T cell proliferation post-transplant and the encouragingly low rates of early, severe aGvHD suggest that costimulation blockade may be an effective agent for aGvHD prophylaxis and support the conduct of a larger, randomized phase 2 study.

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Defining the GvHD Transcriptome: Network Analysis Identifies the Key Pathways Responsible for Primate GvHD Pathogenesis and the Mechanisms of Partial GvHD Control with Sirolimus

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We have developed a systems-biology approach to studying GvHD, using whole-transcriptome analysis of pathogenic T cells. Using computational methods, we have identified, for the first time, the transcriptional networks that drive primate GvHD, and that lead to its partial control through mTOR inhibition with sirolimus.

Methods: CD3+/CD20- T cells were purified flow cytometrically from 4 cohorts: (1) Healthy Controls (HC n = 15); (2) Auto-HSCT recipients (n = 3); (3) Untreated Allo-HSCT recipients who developed severe GvHD, (GvHD n = 4); and (4) Allo-HSCT recipients receiving sirolimus alone (Sirolimus n = 4). Purification of T cells and RNA was followed by primate-specific Affymetrix Gene Array analysis.

Computation: Gene array signals were processed using the Robust Multichip Averaging Method. Principal Component Analysis (PCA) revealed that variation was primarily determined by the experimental cohort (Figure 1A). This result was critical, and confirmed that transcriptomics could be applied to identify genes and pathways controlling GvHD. Differentially expressed genes (DE) were defined, yielding unique and overlapping gene signatures, with 775 DE genes between GvHD and HC and 286 DE genes between Sirolimus and HC (Figure 1B). Finally, using Ingenuity Pathway Analysis (IPA) we characterized gene signatures according to molecular pathways (Figure 1C).

Results: T cells from animals with severe aGvHD showed transcriptional signs of rampant proliferation and cytotoxicity, but also of cell death. IPA identified highly statistically significant upregulation of Cell Cycle as well as Cell Trafficking and Inflammatory Response networks (Figure 1C, $P <$ .001) These networks contained some expected genes and some surprises. Thus, GvHD was associated with upregulation of JAK and IFN signaling ($P <$ .001), but unexpectedly, was also associated with upregulation of the Sonic Hedgehog